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Biofilms are formed by a matrix that creates a regulated environment for microbial communities that allows the passage of nutrients, gases and water and restricts the entrance of harmful substances. Biofilm formation is dependent on the environment in which the bacterial cells are in their planktonic form. During biofilm development enzyme systems are activated, including proteases. Another aspect of bacterial survival is the accumulation of polyhydroxyalkanoates (PHA). *Bacillus subtilis* (bacterium considered as generally recognized as safe (GRAS) by the Food and Drug Administration) is able to produce biofilms free of endotoxins and accumulate PHA. In this work it was studied the production of proteolytic enzymes in different culture media and their relationship with the formation of biofilms, spores and PHA synthesis. *B. subtilis* free of plasmids innoculum was one percent (v/v) of a 12h growth at 30°C in nutritive agar. The culture media were nutritive broth with glucose, mannitol or xylose. It was also studied the effect of different inorganic nitrogen sources. The biofilm formation was determined in static conditions at 48 h incubation. Protease activity was measured with azocaseine. The sporulation rate was determined as the number of viable cells present after a treatment at80°C for 20 min. The PHA was determined by fluorescence with Nile Blue and by crotonic acid formation. Data were analyzed by ANOVA test. The proteolytic activity of the liquid medium under the biofilm was dependent on the carbon source, being higher for xylose and mannitol than for glucose (87; 70 and 11 protease units, respectively). This activity was directly correlated with the production of biofilm (0.27; 0.20 and 0.13 mg biofilm/ml incubated) and spore formation in the biofilm (2,0E+07; 6,2E+04 and 6,0E+02 cfu/mg biofilm). Instead, the protease activity was inverse to PHA accumulation in the biofilm (48; 79 and 132 µg PHA/mg biofilm). Using mannitol as carbon source, the presence of inorganic nitrogen (ammonium and nitrate) decreased proteolytic activity in the static liquid media (12 and 15 units protease, respectively) and the biofilm formation (0.13 and 0.17 mg biofilm/ml incubated); nitrate but not ammonium increased PHA accumulation in the biofilm (115 and 78 µg PHA/mg biofilm). These results suggest that the proteolytic activity could have an important role in bacterial biofilm production, being directly related to spores formation and inversely with PHA accumulation. This might suggest that the *B. subtilis* in the biofilms from diverse culture media could favored the generation of bacterial structures in the biofilm with different capacities to survive in the environment, according to the spore formation and the PHA accumulation in the sessile cells.

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ISOLATION AND USE OF EXTREMOPHILES IN THE DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND IN SALINE WASTEWATER

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Biochemical oxygen demand (BOD) is a useful parameter for assessing the biodegradability of dissolved organic matter in water. At the same time, this parameter is used to evaluate the efficiency with which certain processes remove biodegradable natural organic matter. However, the values of BOD in saline water are very low because NaCl destroy the seed. Therefore the incorporation of a suitable selection of bacteria (which is not always easy to obtain) is required. In this sense the extremophile bacteria, particularly halophilic bacteria or halophytes can contribute to the development of this technique. The objective of this work was to develop a BOD analysis protocol for the analysis of saline effluent. Within this context a halophilic microorganisms was isolated from Salinas del Bebedero, San Luis, Argentina. Salt sample was cultured in agar Dussault and Lachance. Subsequently, the isolated colonies were cultured in medium Dussault and Lachance broth for 7 days at 37ºC with constant stirring (180 rpm). Subsequently the nucleic acid extraction method was performed by Brosius et al., based on the lysis of the cells with glass beads, and 10% SDS. To amplify the variable region V3-V5, 16S rDNA primers described as F344-R915 (Stahl et al, 1991) were used: 344F: 5'- ACG GGG CGC YGCAGCAGG GA-3 'and 915R: 5'- GTG CTC CCC CGCCAATTC CT-3 '; *Halorubrum salsolis* DNA was used as control. The size of the reaction products was characterized on agarose gel 1% and evaluated on an image analyzer. The molecular weight was determined by comparison with molecular weight marker 100 bp DNA Ladder (Promega). The PCR fragments were sequenced by Macrogen (Korea), with Reaction Kit READY PRIMS a model ABI prisma373A sequencer (PE AppliedBiosystems). The sequencing results were analyzed by comparison with 16S rRNA genes databases (GenBank). The organism under study under study showed a 100% identity to gender *Haloarcula sp*. One milliliter of the strain (10^6 cell per mL) was used as seed in the BOD analysis protocol. Standard solution of glucose-glutamic acid (BOD = 20 mg L^{-1}) with different concentrations of NaCl (5%, 10%, 20%, 30% and 40%) were used. BOD bottles were incubated at 20 °C for 5 days. No significant differences were found among differents samples, since their organic matter content was similar (CV <2.1%). In conclusion, *Haloarcula sp* can be used in the determination of BOD in saline effluent.